

DEFENSE AGAINST BIODEGRADATION OF MILITARY MATERIEL

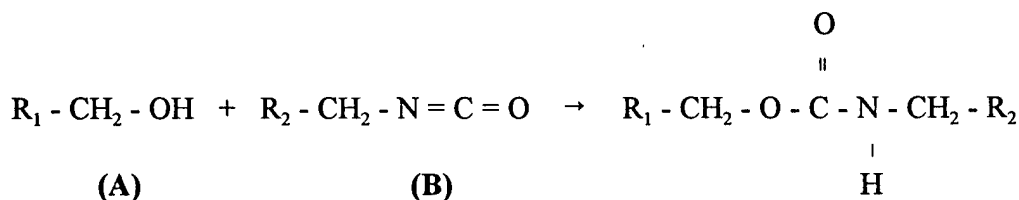
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Non-lethal weapons represent novel threats to warfighters, requiring equally novel defensive measures. In addition to direct anti-personnel applications, such weapons may be designed specifically to degrade military forces' mobility, logistical support and equipment maintenance programs in a clandestine manner prior to or during military engagements, in a time frame of minutes to weeks. Such systems, patterned after microorganisms and their products, can be directed at non-living targets such as highway and runway surfaces, metal parts and coatings of weapons, support equipment and vehicles, fuels and other supplies and replacement parts. Microbial-derived systems may be used to accelerate the corrosion, degradation or decomposition of roads and aircraft runways. In addition, targeted deterioration of metal parts, coatings and lubricants of weapons, vehicles and support equipment, as well as fuels and other supplies, would significantly increase the cost and logistical burden of sustaining military operations. An important threat area addressed by this research includes denial of land areas to vehicles and aircraft by reduction of terrain trafficability and vehicle operation. Another very real threat involves the ability to disable or neutralize equipment and facilities, by degrading fuels and other supplies, and increasing maintenance requirements.

Nature has provided many examples of natural degradation by microorganisms of metals (1-4), fuels (5,6) and a variety of synthetic products (7,8), as well as structures and systems that incorporate or depend on such products. An example of a military material that such weapons may target is the synthetic high-strength polymer, Kevlar, or novel biomimetics of Kevlar based on spider silk. Asphalt is degraded by several strains of bacteria, leading to greatly reduced road surface lifetimes (9). Components of asphalt used for other construction purposes also suffer failure as a direct result of bacterial degradation (10). Cement is subject to rapid, component-specific attack by microbes (11). Most classes of paints and coatings are also vulnerable to degradation by microbial products (12-14). Virtually all petroleum, oil and lubricants (POL) of military relevance are vulnerable to degradation by microbial action (5). Many microorganisms also naturally produce minute granules called inclusion bodies that are made of salt crystals, metals or plastic-like compounds (polyhydroxyalkanates). These particles will quickly clog high efficiency filters, and convert critical lubricants of weapon systems into gums or abrasives. The initial phase of our research has focused on identifying and characterizing the degradative potential of products from naturally-occurring microorganisms. This work has been extended to the development of model microbial systems using genetically modified microorganisms (GEM) that express focused degradative capabilities. These will be further modified to be self-limiting, either by incorporation of timed "suicide" genes, or other alterations that prevent their persistence in the environment beyond pre-determined limits of space or time. The natural and model microbial systems are being studied to understand the enzymatic and other mechanisms by which

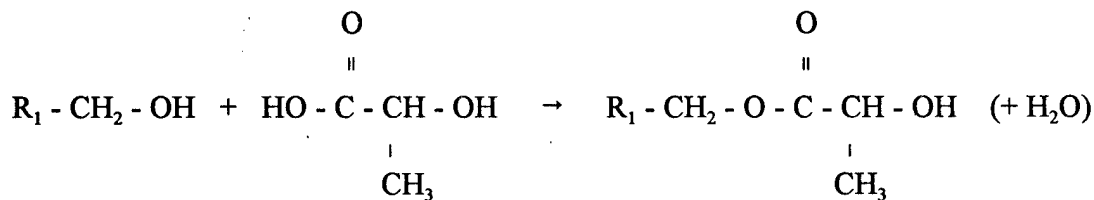
degradation of materials is effected. The knowledge of microbial degradation pathways gained in this study will be used to develop biomimetic chemical systems that reproduce the specific degradative capabilities, but without the requirement for living microorganisms. The genetic engineering techniques employed are standard laboratory practices, requiring no special isolation laboratories, and this materials science research is not restricted in any way by the 1972 Geneva Convention on Biological Warfare or any other international agreement.

The second phase of the research will focus on devising defensive measures, or "vaccination" strategies, to protect our military materiel against offensive actions that employ biodegrading microbes or their products. Extracellular enzymes from bacteria or fungi can easily be produced in large quantities, and potentially deployed for such purposes. For example, it is quite possible that microbial derived or based esterases might be used to strip signature-control coatings from aircraft, thus facilitating the detection and destruction of the aircraft. Countermeasures should be developed well in advance of need. One example of such "vaccination," involving the protection of polyurethane paints and coating, is described below. Naval ships and aircraft use polyurethane coatings in a variety of applications to protect surfaces from corrosion. Polyurethane is vulnerable to enzymatic degradation by a number of naturally occurring microorganisms (12,13,15,16). Current Navy aircraft coatings are two-component polyurethanes and are described in military specification MIL-PRF-85285C. The first component contains a polyol resin **A**, pigments, and other ingredients. The second component contains a polyisocyanate **B**. The curing reaction is shown in Scheme 1:

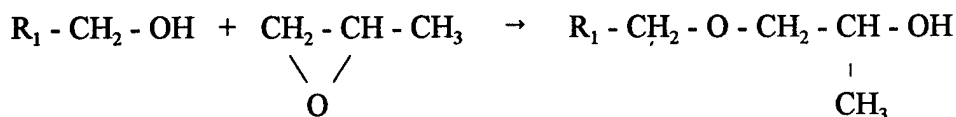


Scheme 1

To ensure rapid and complete reaction, and to keep the price of the starting materials comparatively low, primary isocyanates and primary alcohols are used as shown in Scheme 1. The sterically-unhindered urethane linkage thus produced is easily attacked and severed by esterases. Chemical groups that restrict or prohibit access of the enzyme to the urethane group confer stability against enzymic attack. Thus, the polyol of Scheme 1 could easily be modified as shown in Schemes 2 and 3 below:



Scheme 2



Scheme 3

In Scheme 2, each primary hydroxyl group in the polyol is reacted with lactic acid to produce a terminal secondary alcohol. In Scheme 3, the polyol is modified with propylene oxide, which also creates secondary alcohol groups. Reaction of either of these modified polyols with isocyanate will produce a polyurethane coating essentially identical to that obtained from the unmodified polyol. However the coating will now contain blocked urethane groups that are "immune" to enzymatic attack.

There are some drawbacks to this approach. Secondary alcohols react slower than primary alcohols, so the curing time of the coating would be lengthened, or else the catalyst level would need to be raised. As a result, costs would also be increased.

Previous work at the Naval Research Laboratory (NRL) identified and produced in the laboratory an enzyme from a naturally occurring fungus, which rapidly decomposes polyurethane (15). This work was subsequently extended at NRL, to create a new genetically engineered microorganism that overproduces the polyurethane degrading enzyme (U.S. Patent, Navy Case No.75461) (16).

All of the armed services in a joint military operation will benefit from technology that protects the warfighters' overall ability to initiate and sustain combat operations. Vaccinating aircraft runway surfaces allows U.S. Air Forces to sustain operations to control the skies over enemy territory. Protecting road and highway surfaces supports the mobility of Army and Marine land forces, particularly troop and supply transport. Protecting fuels, replacement parts and other supplies that support a war effort gives an advantage to all branches of our military by enhancing logistical support systems. The potential for clandestine employment of these non-lethal weapon systems, particularly since their effects in many cases may closely mimic natural processes, gives an adversary the added advantage of deniability. For this reason, defensive measures must be proactive, rather than reactive.

In addition, characterization of degradative mechanisms and development of "vaccination" strategies will have significant dual use applications in protecting military and commercial materials and materiel from naturally-occurring biodegradation problems, as well as from offensive military and terrorist attacks of this nature. Scientific expertise capable of developing anti-materiel technology patterned after microbial systems unquestionably is already present in the laboratories of potential adversary states, and the likelihood of near-term development of such threats is great. Failure to counter this threat with a focused research program jeopardizes the warfighting capability of the U.S. and its allies.

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